REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and the following remarks.

I. CLAIM STATUS IN AMENDMENTS

Claims 1-24 are pending in this application when last examined and stand rejected.

Claims 1-3 have been canceled without prejudice or disclaimer thereto. Applicants reserve the right to file a continuation or divisional application on any canceled subject matter.

Claim 4 is amended to delete the phrase "such as". Support can be found in the claim as filed and in the disclosure, for example, in examples 2-3 on pages 7-9. Claim 4 is further amended to clarify that the DNA vector replicates in the prokaryotic or eukaryotic cells prior to exposure to a mutagenic agent as supported by the disclosure, for instance, at page 6, line 23 to page 7, line 10, and original claim 4.

Claim 10 is amended to replace the phrase "high identity degree" with "having at least 99.5% identity." Support can be found in the disclosure, for example, at page 12, lines 21-22.

Claims 4-24 are also amended to improve the language therein to better conform to U.S. practice and English grammar form. Such revisions are non-substantive and not intended to

narrow the scope of protection. Support for such can be found in the claims as filed.

No new matter has been added by the above claim amendments.

Claims 4-24 are pending upon entering this amendment.

II. INDEFINITENESS REJECTIONS

Claims 1-24 were rejected under 35 USC §112, second paragraph, as being indefinite for the reasons set forth on pages 2-3 of the Office Action.

It is respectfully submitted that the present amendment overcomes this rejection.

To start, claims 1-3 have been canceled without prejudice or disclaimer thereto.

Claim 4 has been amended to remove the phrase "such as".

Claim 10 was also amended to replace the term "high identity degree" with the phrase "having at least 99.5% identity", as supported by the disclosure at page 12, lines 21-22. **In doing so,** claim 10 has been amended along the lines suggested by the Examiner at page 3 of the Office Action.

In view of the above, the §112, second paragraph indefinitness rejections are untenable and should be withdrawn.

III. §101 REJECTIONS

On page 2 of the Office Action, claims 1-3 are rejected under 35 USC §101 on the basis that they fail to recite proper process steps. The present amendment overcomes this rejection, as claims 1-3 have been canceled without prejudice or disclaimer thereto. Withdrawal of the rejection is therefore solicited.

IV. ANTICIPATION REJECTION

On page 4 of the Office Action, claims 4-6, 10-14, 16-18 and 21-22 were rejected under 35 USC §102(b) as anticipated by HINDS et al.

This rejection is respectfully traversed.

To start, it is noted that the present invention concerns the use of Applicants' new finding that a replication competent DNA molecule treated with a mutagenic substance which blocks DNA replication, when transformed into a prokaryotic or eukaryotic cell undergoes much higher levels of homologous recombination than an untreated DNA molecule, and so is a far superior agent for transforming prokaryotic or eukaryoptic cells by targeted homologous recombination. The current application provides and claims a method (claims 4-24) according to the current invention which overcomes limitations associated with prior art methods of transforming a prokaryotic or eukaryotic cell by targeted homologous recombination.

To anticipate, a cited prior art reference must either expressly or inherently teach each and every element of the claimed invention. See MPEP § 2131.01.

The present invention as represented by independent claim 4 calls for a method for *in vitro* insertion of a nucleic acid of interest initially included in a DNA vector, within a predetermined target nucleotide sequence present in a chromosome contained in a prokaryotic or eukaryotic cell, said method comprises:

a) contacting the DNA vector comprising the nucleic acid of interest, and replicating said DNA vector in said prokaryotic or eukaryotic cell, with a mutagenic agent blocking the DNA replication in the cell;

b) transfecting said prokaryotic or eukaryotic cells with the DNA vector obtained at the end of step a); and

c) selecting prokaryotic or eukaryotic cells for which the nucleic acid of interest has been integrated into the predetermined target nucleotide sequence.

Applicants respectfully submit that HINDS et al. fails to disclose or suggest each and every element of independent claim 4, and therefore, HINDS et al. cannot anticipate the claimed invention.

In this regard, HINDS et al. relates to the use of non-replication competent DNA molecules namely, suicide plasmids, single stranded DNA, and phagemids.

It is noted that a suicide plasmid is one which, due to a number of different mechanisms, cannot replicate in a host cell following transfection and which comprises a selectable marker gene. This means that selection of cells transfected with the suicide plasmid results in all selected clones having a genomic insertion of the selectable marker gene and associated sequences.

A phagemid or phasmid is a type of cloning vector developed as a co-infection of the M13 helper phage and plasmids to produce a smaller version of the virus. Phagemids contain an origin for double stranded replication as well as an origin for single stranded replication, mostly not comprising the entire phagemid (*i.e.*, only a small part of the phagemid is copied as a single strand), but not all the necessary viral genes to replicate. This means that the presence of a "helper" virus such as f1, is required to provide the rest of the genes necessary to replicate viral proteins to thereby allow more virus particles to be created using the host cell's resources. Without this "helper" virus, however, the phagemid is incapable of independent replication.

In contrast to the above, the method of the present invention as shown in independent claim 4 relates exclusively to methods in which the DNA vector molecule replicates in the prokaryotic or eukaryotic target cell prior to exposure to a mutagenic agent. See step (a) of independent claim 4, which calls for: "a) contacting the DNA vector comprising the nucleic

acid of interest, and replicating said DNA vector in said prokaryotic or eukaryotic cell, with a mutagenic agent blocking the DNA replication in the cell." [Emphasis added.] See also the description at page 6, line 23 to page 7, line 10 of the specification.

The fact that the DNA vector of interest used in the method of the present invention can replicate inside the target cell is also clear from the examples in the specification regarding transformation of prokaryotic or eukaryotic cells. See, for instance, the description at pages 7-9. For the prokaryotic model (page 7, paragraph 119-137), the nucleic acid of interest comprises an *E. coli* replication origin (paragraph 119), which is used to transform *E. coli* cells (paragraph 13 1-132). Likewise in the eukaryotic model (page 8, paragraph 138-160), the nucleic acid of interest comprises an Epstein-Barr replication origin (paragraph 148) which allows this nucleic acid of interest to replicate a number of times in a human eukaryotic cell transformed by this nucleic acid molecule.

Based on such disclosure, it is clear that the method of independent claim 4, and each of dependent claims 5-24 (which directly or indirectly depend), require using a nucleic acid of interest, which in a first step can replicate in the target prokaryotic or eukaryotic cell.

HINDS et al. fail to disclose or suggest this aspect of the claimed invention. Thus, it is clear that the method of the present invention clearly differs from HINDS et al. For this reason, HINDS et al. cannot anticipate the claimed invention. Consequently, the method of the present invention is clearly novel over HINDS et al.

In addition, it is respectfully submitted that claims 4-24 are also unobvious over the teaching of HINDS et al. First, as acknowledged in HINDS et al. (page 525, Discussion section, 1st paragraph), the transformation of slow growing Mycobacteria is known to be difficult and generalizations from other prokaryotic organisms to Mycobacteria have most often not been shown to be useful. Likewise methods known to work in Mycobacteria are not generally applicable to other prokaryotes. Based on this understanding, one of ordinary skill in the art, upon reading this reference and in view of the knowledge in the art, wishing to develop a method to improve the transformation of both prokaryotic and eukaryotic organisms by homologous recombination would not have a reasonable expectation of success using the technique in HINDS et al. shown to work in a limited number of Mycobacteria species to a variable extent for a number of target genes. It simply would not have been unpredictable to do so to arrive at the present invention given the disclosure in HINDS et al.

Second, the technique described in HINDS et al. uses exclusively non-replication competent DNA molecules. The use of such "suicide" vectors in prior art homologous transformation methods is a key technical feature of such methods as the rate of homologous recombination events is orders of magnitude less than that of the transformation of an organism by a plasmid and so the detection of homologous recombinants would be entirely impractical if the DNA vector was able to reproduce within the prokaryotic target cell. Starting with the technique of HINDS et al., therefore, the skilled artisan seeking to develop a new technique to improve the transformation of both prokaryotic and eukaryotic organisms by homologous recombination, would have continued to use "suicide" vectors and not the replication competent vectors of the method according to claim 4 of the present application.

The use of such replication competent vectors in the current invention is one important technical advantage over the method in the prior art. It allows the materials used in the method according to the present invention to be far more easily generated, maintained and modified using standard molecular biology techniques.

In view of the above, it is clear that the technique disclosed in HINDS et al. would not have been reasonably predictive of the claimed invention. As such, it is respectfully

submitted that the invention of claims 4-24 are both novel and unobvious over HINDS et al.

Therefore, the above §102(b) anticipation rejection over HINDS et al. is untenable and should be withdrawn.

On pages 4-5 of the Office Action, claims 4-5, 10-14, 16-19, 21 and 23 were rejected under 35 USC §102(b) as anticipated by GANIATSAS et al.

This rejection is respectfully traversed.

Applicants respectfully submit that, like HINDS et al., GANIATSAS et al. also fails to disclose or suggest each and every element of the present invention. Accordingly, GANIATSAS et al. cannot anticipate any aspect of the present invention.

To start, GANIATSAS et al. relates to an entirely different methodology. In particular, GANIATSAS et al. is not concerned with a study of the effects of UV upon homologous recombination mediated transformation events.

Instead, GANIATSAS et al. relates to a study into the mitogen-activated protein kinase activator SEK1. The authors studied SEK1 *in vivo* by treating SEK1 competent/incompetent cells with various activating stimuli such as interleukin 1, tumour necrosis factor α, heat shock, UV irradiation and protein synthesis inhibition (page 6881 paragraph 1. sentence 2). SEKI activates in response to these stimuli and particularly in response to UV (page 6881 paragraph 2, sentence 3).

It is correct that the SEK1 gene was targeted in an ES cell line using a homologous recombination strategy so as to generate $SEK1^-$ lines for comparison with $SEK1^+$ lines. The genotypes -/- and +/+ were then compared by western blot following exposure to a stimulus such as UV or heat shock (Figure 2, sentence (b)).

However, this method in GANIATSAS et al. is not the same transformation technique as the one described and claimed in the current application. In particular, the stimulus (be it heat shock or UV exposure) in GANIATSAS et al. is performed after the alteration of the genome of the ES cell line and the selection and propagation of the altered cell line to a sufficient density so as to allow western blot analysis to be performed. The alteration described in GANIATSAS et al., therefore, corresponds to a prior art homologous recombination mediated gene inactivation technique.

This method differs substantially from the methodology of the present invention.

The current invention specifically seeks to overcome the limitations of such prior art techniques (like those of GANIATSAS et al.) by treating the nucleic acid of interest, which will be used in the transformation method, with a mutagenic agent which blocks the DNA replication mechanism of the host cell, and then using this treated nucleic acid molecule of interest as the agent of transformation to transform the cells. Please see step

(b) of independent claim 4, which states "b) transfecting prokaryotic or eukaryotic cells with the DNA vector obtained at the end of step a)." [Emphasis added.]

Also, please keep in mind that the DNA vector of step (a) has been treated with a mutagenic agent.

Accordingly, the invention of claim 4, and each of dependent claims 5-24 (which depend either directly or indirectly thereon), require the treatment of the nucleic acid of interest prior to its use in the transformation of a target prokaryotic or eukaryotic cell. This method is clearly novel over GANIATSAS et al.

In addition, Applicants respectfully submit that the method of the present invention is unobvious over GANIATSAS et al. One of ordinary skill in the art, upon reading the reference, would have no reason to modify the prior art homologous recombination technique disclosed therein, since by using such technique, the altered cell lines were prepared with which GANIATSAS et al. successfully used to investigate SEK1. Accordingly, there was no suggestion and/or motivation to alter the methodology in GANIATSAS et al. to arrive at the claimed invention.

Likewise, it is respectfully submitted that no combination of the teachings of GANIATSAS et al. and HINDS et al. would result in method of claim 4 of the current application. This is so, because neither reference uses a replication

competent DNA molecule as the agent of transformation as in the present invention. GANIATSAS et al. uses the prior art pGKneo/TK construct as the agent of transformation. This well known vector is not a replication competent vector in target cells.

In view of the above, Applicants submit that the invention of claims 4-24 is both novel and unobvious over GANIATSAS et al. alone or in combination with other cited references.

Therefore, the above §102(b) anticipation rejection over GANIATSAS et al. is untenable and should be withdrawn.

On pages 5-6 of the Office Action, claims 4-21 and 23-24 were rejected under 35 USC §102(e) as anticipated by HOEIJMAKERS et al.

This rejection is respectfully traversed.

Applicants respectfully submit that this reference also fails to disclose or suggest each and every element of the claimed invention.

HOEIJMAKERS et al. relates to a different technical problem and describes a method of determining the effects of an agent upon the integrity of DNA in a eukaryotic cell. In particular, HOEIJMAKERS et al. does this by monitoring the levels of HR23 protein binding molecules in cells following exposure to a DNA modifying agent. The HR23 protein binding molecules investigated include XPC, MAG, CREB and p53.

The cells used in this method were engineered so as to have altered expression of HR23A and/or HR23B. This was achieved by targeted gene disruption in accordance with the scheme shown in Figure 1 of HOEIJMAKERS et al. The strategy outlined in Figure 1 (and described at page 9, paragraphs 108-115 of HOEIJMAKERS et al.) corresponds to a prior art homologous recombination gene alteration strategy.

The exposure of the altered and unaltered cells to a DNA lesion inducing agent such as UV, occurs only once suitably and stably transformed cells have been created, selected and propagated into whole animals from which further samples can be derived.

The present invention differs from this prior art technique as taught in HOEIJMAKERS et al. As indicated above, the current invention specifically seeks to overcome the limitations of such prior art techniques by treating the nucleic acid of interest which will be used in the transformation method with a mutagenic agent which blocks the DNA replication mechanism of the host cell and using this treated nucleic acid molecule as the agent of transformation. Please see the above arguments concerning step (b) of claim 4 as evidence that this element is a requirement of the method according to claim 4.

Again, claim 4, and each of dependent claims 5-24 (which depend either directly or indirectly thereon), specify that the method involves the treatment of the nucleic acid of

interest prior to its use in the transformation of a target prokaryotic or eukaryotic cell. HOEIJMAKERS et al. fails to disclose or suggest this feature of the present invention. Therefore, the present invention is clearly novel over HOEIJMAKERS et al.

In addition, Applicants respectfully submit that claim 4, and each of dependent claims 5-24, are also unobvious over HOEIJMAKERS et al. The skilled artisan, having read HOEIJMAKERS et al., would have no reason to modify the prior art homologous recombination technique disclosed therein to arrive at the claimed invention, since it was with this technique that the altered cell lines were prepared with which HOEIJMAKERS et al. successfully developed their new method to monitor DNA integrity following exposure to a mutagenic agent. Accordingly, there was no suggestion and/or motivation to alter/modify the methodology in HOEIJMAKERS et al. to arrive at the claimed invention.

Likewise, it is respectfully submitted that no combination of the teachings of HOEIJMAKERS et al. and HINDS et al. would result in the method of claim 4 of the current application. In this regard, neither reference uses a replication competent DNA molecule as the agent of transformation described. HOEIJMAKERS et al. uses the prior art pGKneo/TK construct as the agent of transformation. This well known vector is not replication competent in target cells.

For these reasons, the present invention is both novel and unobvious over HOEIJMAKERS et al.

Therefore, the above §102(e) anticipation rejection over HOEIJMAKERS et al. is untenable and should be withdrawn.

V. CONCLUSION

In view of the foregoing amendments and remarks, it is respectfully submitted that the present application is in condition for allowance and early notice of to that effect is hereby requested.

If the Examiner has any comments or proposal for expediting prosecution, please contact the undersigned attorney at the telephone number below.

The Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

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